

Attorney Docket No.: ISPH-0537  
Inventors: Dean et al.  
Serial No.: 09/800,629  
Filing Date: March 7, 2001  
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A1  
[Oligonucleotides were tested in EL-4 T cells (ATCC TIB-39, American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110) by Northern blot analysis as described in previous examples using a commercially available murine IL-5 probe. These cells are PHA responsive and PMA plus CAMP elevating agents induce a several hundredfold increase in IL-5 synthesis by these cells. Cells were maintained and stimulated to express IL-5 according to published methods and transfected with oligonucleotide via electroporation.]--

At page 66, starting at line 22, please replace the paragraph with the following paragraph:

A2  
[These oligonucleotides were electroporated into human HSB-2 cells and tested for effect on IL-5 mRNA by Northern blot analysis as described in previous examples. The HSB-2 T-cell line was obtained from the American Type Culture Collection (10801 University Boulevard, Manassas, VA 20110) and cells are cultured according to ATCC recommendations. They produce IL-5 upon induction with PMA + ionomycin. Oligonucleotides were tested by Northern blot analysis at a concentration of 10  $\mu$ M for their ability to block IL-5 mRNA expression. The results are shown in Table 5.]

At page 69, starting at line 19, please replace the paragraph with the following paragraph:

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A3  
Using an RNase protection assay (RiboquantJ hCK4, Pharmingen, La Jolla, CA), it was determined that ISIS-16085 inhibited IL-5 expression in a second T cell line, CEM (obtained from American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110) with an IC50 estimated at approximately 25  $\mu$ M. IL-5 expression is induced in these cells by treatment with PMA plus ionomycin in the presence of IL-2, anti-CD28 cross-linking antibody, and dibutyryl cAMP. Dose response analysis of ISIS 16085 vs. Its 5-mismatch control in stimulated CEM cells showed a dose-dependent decrease in IL-5 mRNA of about 50% at 25  $\mu$ M oligonucleotide, compared with about 22% reduction with the mismatch control. No decreases were seen in other cytokine gene products measured in this assay.

At page 73, starting at line 3, replace the paragraph with the following paragraph:

A4  
--Murine BCL cells were chosen for screening antisense oligonucleotides targeted to murine IL-5 receptor- $\alpha$ . These are B-cell leukemia cells derived from a spontaneously arising tumor of BALB/c origin, and proliferate in response to murine or human IL-5. This is a CD5+ line which resembles a subset of human chronic lymphocytic leukemia tumors and secretes IgM upon lipopolysaccharide stimulation. Cells were obtained from the